Exploring the Regional Characteristics of Intestinal Drug Metabolism and Fibrogenesis

Iswandana, Raditya

Chapter 5

Regional Differences in Human Intestinal Drug Metabolism

R. Iswandana[a,b], M.I. Irianti[a,b], D. Oosterhuis[a], H.S. Hofker[c], M.T. Merema[d], M.H. de Jager[d], H.A.M. Mutsaers[a,e], P. Olinga[a,*]

[a]Division of Pharmaceutical Technology and Biopharmacy, Department of Pharmacy, University of Groningen, The Netherlands

[b]Faculty of Pharmacy, Universitas Indonesia, Indonesia

[c]Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands

[d]Division of Pharmacokinetics, Toxicology and Targeting, Department of Pharmacy, University of Groningen, The Netherlands

[e]Department of Clinical Medicine, Aarhus University, Denmark

**Corresponding author*

Drug Metabolism and Disposition **46(12), 1879 – 1885 (2018)**

ABSTRACT

Background

The intestines are key for the absorption of nutrients and water as well as drug metabolism, and it is well known that there are clear differences in the expression profile of drug metabolism enzymes along the intestinal tract. Yet only a few studies have thoroughly investigated regional differences in human intestinal drug metabolism. In this study, we evaluated phase I and phase II metabolism in matched human ileum and colon precision-cut intestinal slices (PCIS).

Methods

To this end, human PCIS were incubated for 3 h with testosterone and 7-hydroxycoumarin (7-HC) to examine phase I and phase II metabolism, respectively. Metabolite formation was assessed by high-performance liquid chromatography analysis.

Results

Our results demonstrated that androstenedione, 6β-hydroxytestosterone, 2β-hydroxytestosterone, and 7-HC sulfate, were predominantly formed in the ileum, while 15α-hydroxytestosterone and 7-HC glucuronide were mainly produced in the colon. Moreover, we also observed sex differences in phase II metabolite formation, which appeared to be higher in males as compared to females.

Conclusions

Taken together, we demonstrated that phase I metabolism predominantly occurs in ileum PCIS, while phase II metabolism mostly takes place in colon PCIS. Moreover, we revealed that human PCIS can be used to study both regional and sex differences in intestinal metabolism.

Keywords:
Cytochrome P450, gastrointestinal tract, glucuronidation/UDP-glucuronyltransferases/UGT, HPLC, metabolite disposition, sulfate conjugation/sulfotransferases/SULT.

Abbreviations:
15α-TOH, 15α-hydroxytestosterone; 2β-TOH, 2β-hydroxytestosterone; 6β-TOH, 6β-hydroxytestosterone; 7-HC, 7-hydroxycoumarin; CAR, constitutive androstane receptor; CYP, cytochrome P450; DMEs, drug metabolizing enzymes; hPCIS, human precision-cut intestinal slices; HPLC, high-performance liquid chromatography; KHB, Krebs-Henseleit Buffer; PCIS, precision-cut intestinal slices; PCTS, precision-cut tissue slices; PXR, pregnane X receptor; P450, cytochrome P450; SULTs, sulfotransferase; TT, testosterone; UGTs, uridine 5'-diphospho-N-acetylgalactosamine glycosyltransferase.

INTRODUCTION

In addition to the absorption of nutrients and water, the intestines also fulfill an important role in drug metabolism.[1–3] This is illustrated by the high expression level of drug metabolizing enzymes (DMEs) along the intestinal tract.[4,5]

In the human body, xenobiotics are metabolized via various pathways including phase I and phase II metabolism. Phase I reactions are oxidation, reduction, and hydrolysis processes. These chemical reactions change the biological activity of the parent compound rendering it less or completely inactive.[6] In the intestines, the most abundant group of enzymes belong to the cytochrome P450 subfamily 3A. Nonetheless, the expression profile of CYP enzymes differs along the intestinal tract and species differences are apparent.[7] For instance, CYP3A13 is more common in the mouse intestine while CYP3A4/5 is predominantly found in the human small intestine.[7] In addition, it has been demonstrated that the mRNA levels of CYP isoforms diminish along the intestinal tract, with the highest CYP expression in the duodenum and the lowest in the colon.[7,8] In order to study metabolic activity, it is key to use suitable substrates. In the last decades, testosterone (TT[9]; **Figure 1**) and 7-hydroxycoumarin (7-HC[9]; **Figure 1**) have been widely used to examine phase I and phase II metabolism, respectively.[10,11] TT can be used to study phase I metabolism since this hormone is hydroxylated by cytochrome P450 enzymes (CYP3A, CYP2A, and CYP2B).[12–15]

Phase II reactions are conjugation reactions, *e.g.* glucuronidation and sulfation, that improve the solubility of endo- and xenobiotics.[6] 7-HC can be used to examine both reactions,[15] glucuronidation of 7-HC is mediated by uridine 5'-diphospho-N-acetylgalactosamine glycosyltransferase (UGTs), while sulfotransferase (SULTs) catalyze sulfation reactions.[3,16]

Until now, most *in vitro* studies on intestinal metabolism were conducted by using subcellular fractions like microsomes or enterocytes. Using advanced analytical methods, it has been demonstrated that the majority of drug metabolism enzymes can be detected in intestinal microsomes. Moreover, it has been shown that microsomes can be used to investigate enzymatic activity and the intestinal contribution to first pass drug metabolism.[17–19] However, microsomes, lack membrane transporters as well as low abundant enzyme systems and the enzymatic activity in microsomes is highly dependent on the isolation method.[14,20–22]

Recently, it has been demonstrated that precision-cut tissue slices (PCTS) can be used to study intestinal drug metabolism.[23] In this *ex vivo* model, all the different cells that constitute an organ are maintained in their original environment allowing for cell-cell and cell-matrix interactions.[24] In precision-cut intestinal slices (PCIS) the organization of the intestinal villi and microvilli is well conserved.[25] Also, the method is highly efficient, since a large number of slices can be prepared from a small tissue sample, and the slices are relatively easy to process.[14] Here, we studied the regional differences in intestinal metabolism by using matched human ileum and colon PCIS focusing on both phase I and phase II metabolism.

MATERIALS AND METHODS

Chemical

All chemicals were obtained from Sigma (Zwijndrecht, the Netherlands) unless stated otherwise. Stock solutions of testosterone (TT) and 7-hydroxycoumarin (7-HC) were prepared in methanol and stored at -20 °C.

Preparation of intestinal slices

Healthy human ileum and colon tissue was obtained following hemicolectomy for adenocarcinoma (See **Table 1** for patient characteristics). Use of human tissue was approved by the Medical Ethical Committee of the University Medical Centre Groningen (UMCG), according to Dutch legislation and the Code of Conduct for dealing responsibly with human tissue in the context of health research (www.federa.org), refraining the need of written consent for 'further use' of coded-anonymous human tissue. The procedures were carried out in accordance with the experimental protocols approved by the Medical Ethical Committee of the UMCG.

Slicing of precision-cut intestinal slices

Preparation of intestinal slices (PCIS) was carried out according to the protocol of de Graaf *et al.,* 2010.[26] In short, intestinal tissue was cleansed by flushing Krebs-Henseleit Buffer (KHB) through the lumen and subsequently divided into 2-cm segments. Afterwards, intestinal cores were prepared using 3% (w/v) agarose (Sigma-Aldrich, Steinheim, Germany) in 0.9% NaCl at 37 °C and embedded in an agarose core-

embedding unit. Next, PCIS were prepared using a Krumdieck tissue slicer. PCIS had a wet weight of approximately 3 mg, and an estimated thickness of 300-400 µm. Following slicing, PCIS were directly transferred to KHB to prevent loss of viability.

Table 1. Characteristics of human PCIS from the human donors.

Human ID	Gender*	Age
Patient 1	M	64
Patient 2	M	52
Patient 3	M	88
Patient 4	F	79
Patient 5	F	65
Patient 6	M	64
Patient 7	F	81

*M=male; F=female

Incubation of slices

After slicing, PCIS were cultured in 12-well plates containing Williams' Medium E + Glutamax (Gibco, New York, NY) supplemented with 14 mM Glucose (Merck, Darmstadt, Germany), 50 µg/ml gentamycin (Gibco) and 2.5 µg/ml fungizone (amphotericin B; Invitrogen, Paisly, Scotland). Slices were cultured at 37 °C in an 80% O_2/5% CO_2 atmosphere, while horizontally shaken at 90 rpm (amplitude 2 cm). For metabolism experiments, PCIS were incubated with TT (250 µM) or 7-HC (500 µM) for 3 h.

Viability

Viability of PCIS was assessed by measuring the adenosine triphosphate (ATP) content of the slices using an ATP bioluminescence kit (Roche diagnostics, Mannheim, Germany), as previously described.[26] Determined ATP values (picomoles) were normalized to the total amount of protein (micrograms) estimated by the Lowry method (BIO-rad RC DC Protein Assay, Bio Rad, Veenendaal, The Netherlands). Results are shown as relative values compared to the related controls.

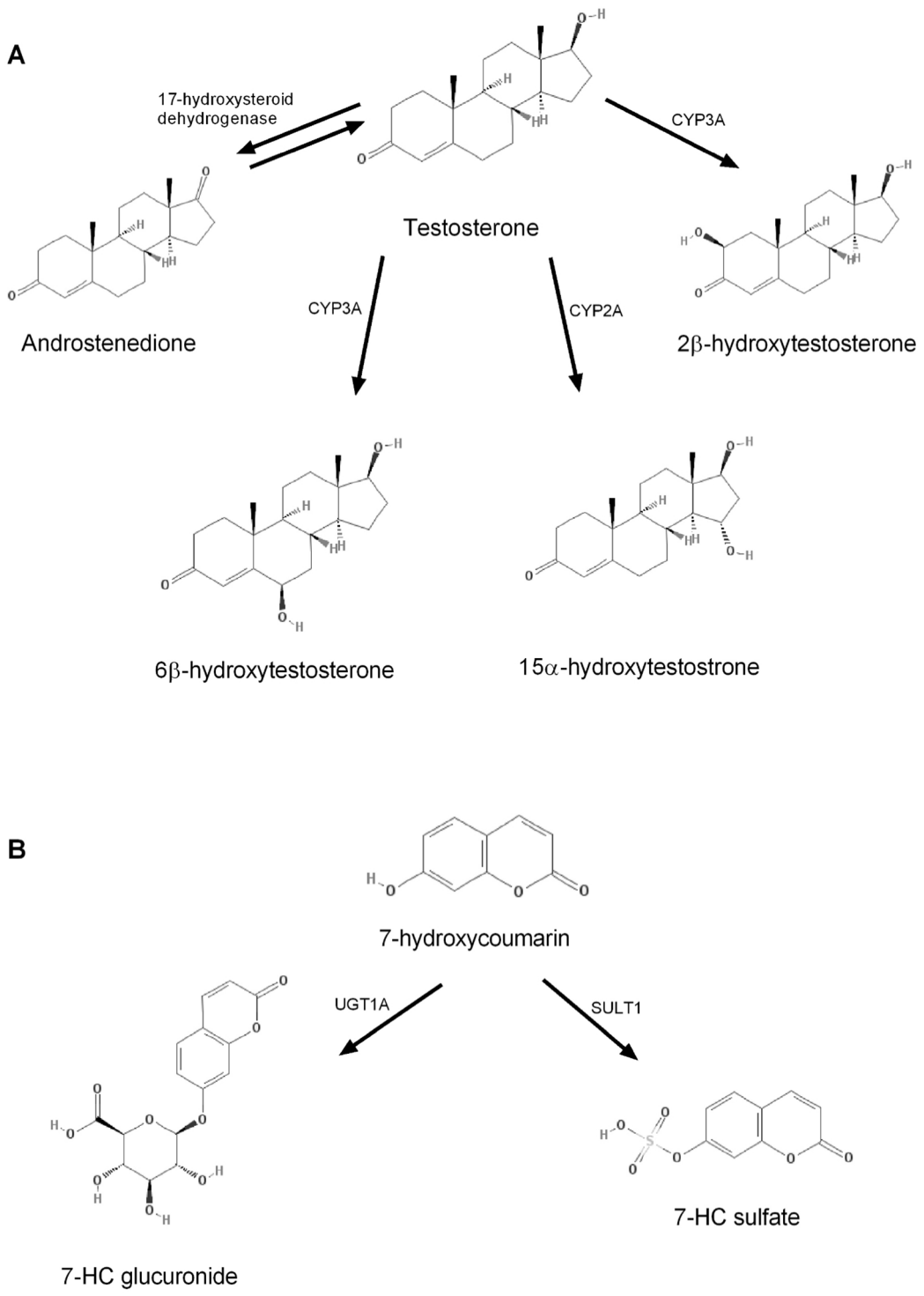

Figure 1. Testosterone and 7-hydroxycoumarin metabolism pathways: (A) Phase I; (B) Phase II.

Metabolite analysis

Testosterone. After incubation with testosterone (TT), PCIS and medium were collected and stored at -20 °C until further use. Sample extraction and high-

performance liquid chromatography (HPLC) analysis was performed as described earlier.[27]

7-Hydroxycoumarin. 7-Hydroxycoumarin (7-HC) and their metabolites do not accumulate in PCIS; therefore the analysis was performed using medium samples only.[10,28] After incubation, samples were collected and stored at -20 °C until use. Levels of 7-HC glucuronide and 7-HC sulfate were determined via HPLC analysis as described previously.[14]

Statistics

Statistics were performed using GraphPad Prism 6.0 via two-tailed Student's t-test and Pearson rank correlation as appropriate. A minimum of three different intestines was used for each experiment, using 3–6 slices from each intestine per condition. The results are expressed as mean ± standard error of the mean (SEM). Statistical differences in ATP levels were determined using the values relative to the control values. Differences between groups were considered to be statistically significant when $P < 0.05$.

RESULTS

Viability of ileum and colon slices

Our laboratory previously demonstrated that rat PCIS can be used to study intestinal metabolism *in vitro*. To gain more insight into human metabolism, we prepared matched ileum and colon PCIS, allowing us to study regional differences in intestinal metabolism. First, we characterized viability of the slices by determining the ATP content. As shown in **Figure 2**, the ATP content of human ileum PCIS significantly increased after 3 h of culture, as compared to the 0-hour control, after which the levels remained stable. Similar results were obtained with colon PCIS, indicating that intestinal slices remain viable in culture for at least 24 h.

Assessment of phase I metabolism in ileum and colon

To study phase I metabolism, we incubated ileum and colon PCIS with TT. As shown in **Figure 3**, several testosterone metabolites were formed in both ileum and colon slices, *e.g.* androstenedione, 6β-hydroxytestosterone (6β-TOH), 15α-

hydroxytestosterone (15α-TOH), and 2β-hydroxytestosterone (2β-TOH). However, there was a clear distinction between the metabolite levels in ileum and colon (**Figure 3**, n=5). Androstenedione, formed by 17β-hydroxysteroid dehydrogenase, is the main TT metabolite that can be found in both the ileum and colon with a mean concentration of 9.55 ± 2.27 (range 3.0-15.6) and 4.34 ± 0.96 (range 2.0-9.3) pmol/μg protein, respectively. 6β-TOH was also found in all patient samples, however, the levels were approximately 4.2-fold higher in the ileum than in the colon (**Figure 3**, n=5). In addition, 2β-TOH levels also appeared to be higher in the ileum than in the colon, with a mean concentration of 0.24 ± 0.11 and 0.06 ± 0.01 pmol/μg protein, respectively, although differences were not statistically significant. Regarding 15α-TOH, no differences were observed. Overall, these results suggest that the ileum plays a bigger role in phase I metabolism as compared to the colon (**Supplementary Table S1**).

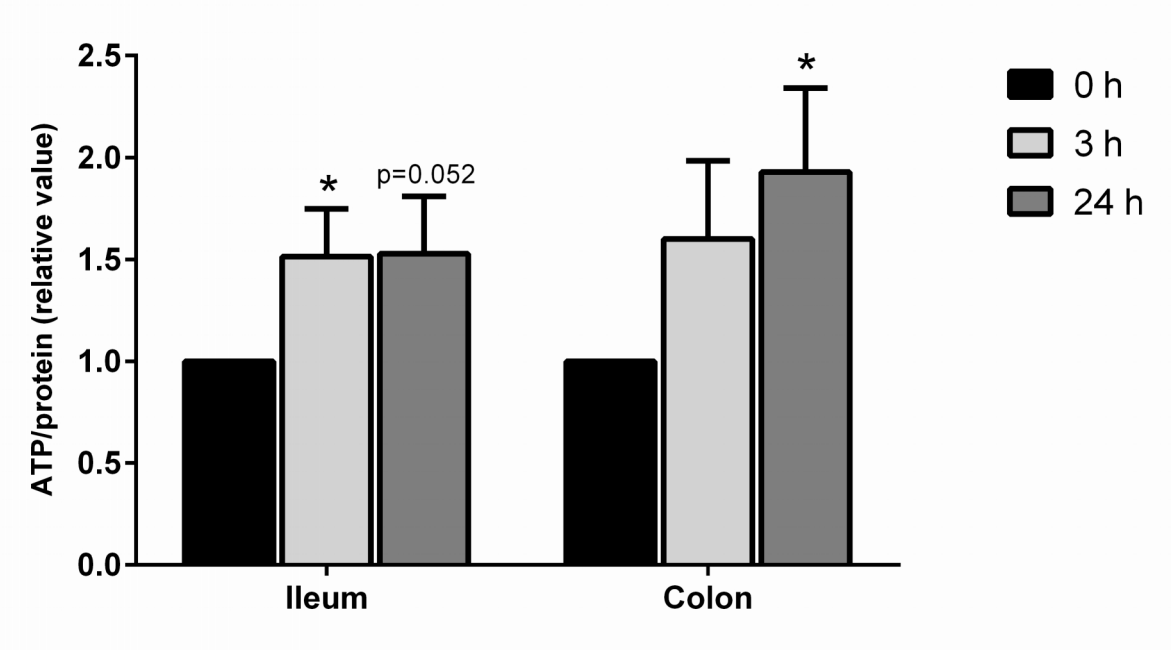

Figure 2. Viability (relative value) of human ileum and colon slices (n=7). Data are expressed as mean +/- SEM. *p<0.05 vs. control (0 h).

Assessment of phase II metabolism in ileum and colon

Next, we investigated phase II metabolism by using 7-HC. As shown in **Figure 4**, both glucuronidation and sulfation reactions took place in the ileum and the colon (n=7). Although not statistically significant, the concentration of 7-HC glucuronide was 2.2-fold higher in the colon as compared to the ileum. In contrast, there was no statistically -significant difference in sulfation in the ileum and colon. Furthermore,

Figure 4 shows that, in the colon, 7-HC glucuronide levels were 5.8 times higher as compared to 7-HC sulfate ($p<0.05$). Taken together, these results suggest that phase II reactions are more prevalent in the colon than the ileum, especially regarding glucuronidation.

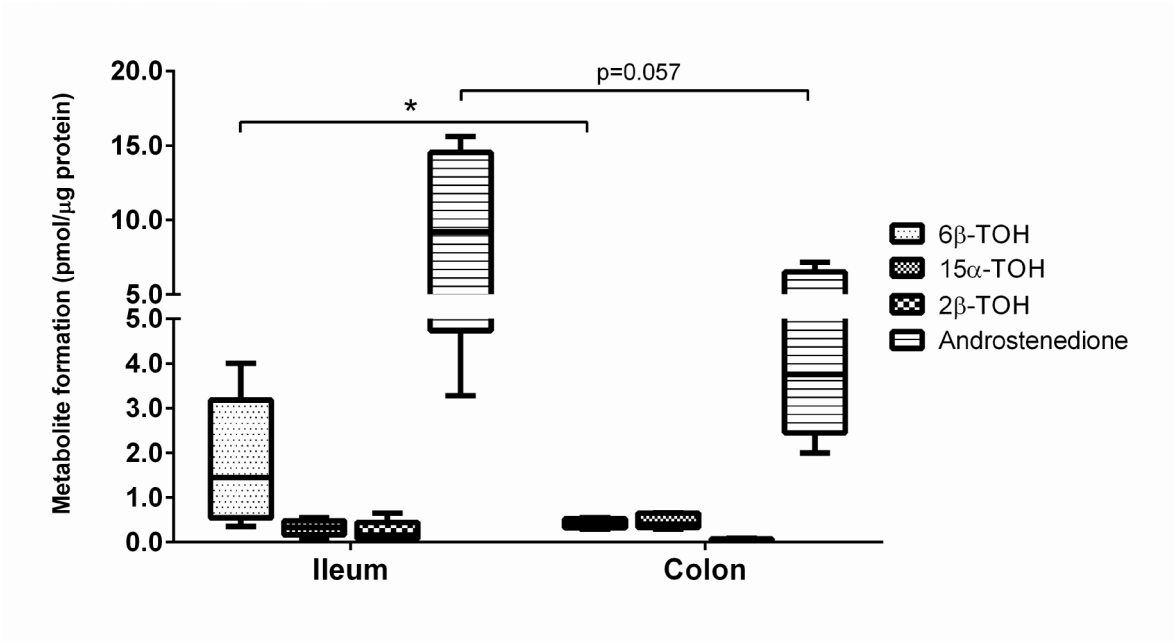

Figure 3. Testosterone metabolite formation in human ileum and colon slices (n=5). Data are expressed as mean +/- range. *$p<0.05$.

Furthermore, we studied the overall occurrence of phase I and II metabolism in the ileum and colon by comparing relative metabolite formation. A ratio higher than one ($r>1$) indicates that the metabolites were more prevalent in the ileum than the colon. Conversely, a ratio of less than 1 ($r<1$) shows that the metabolites were mainly present in the colon instead of the ileum. As shown in **Figure 5**, androstenedione, 6β-TOH, 2β-TOH, and 7-HC sulfate, were predominantly found in the ileum instead of the colon. While, 15α-TOH (0.69), and 7-HC glucuronide (0.45) levels were higher in the colon rather than the ileum (**Supplementary Table 1**).

Moreover, as illustrated in **Figure 6**, we also observed sex differences in phase II metabolite formation, which appeared to be higher in men as compared to women.

Influence of viability on metabolite formation

To exclude the possibility that metabolite formation was influenced by the viability of the slices we performed a correlation analysis, which revealed no significant

correlation between the ATP content of the slices and the formation of most phase I and phase II metabolites, both in the ileum and colon. However, we found a trend towards a negative correlation between ATP levels and 7-HC glucuronide in the ileum (p=0.08, r=0.7).

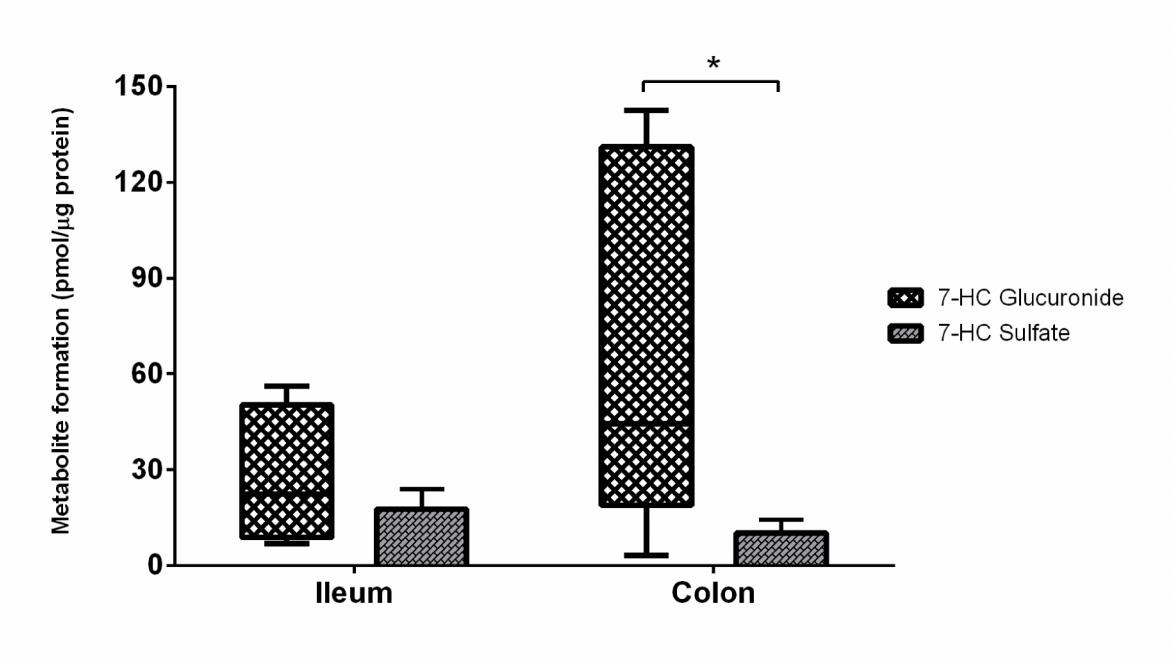

Figure 4. 7-Hydroxycoumarin metabolite formation in human ileum and colon slices (n=7). Data are expressed as mean +/- SEM. *p<0.05. Zero metabolite formation due to the limit of detection of the HPLC.

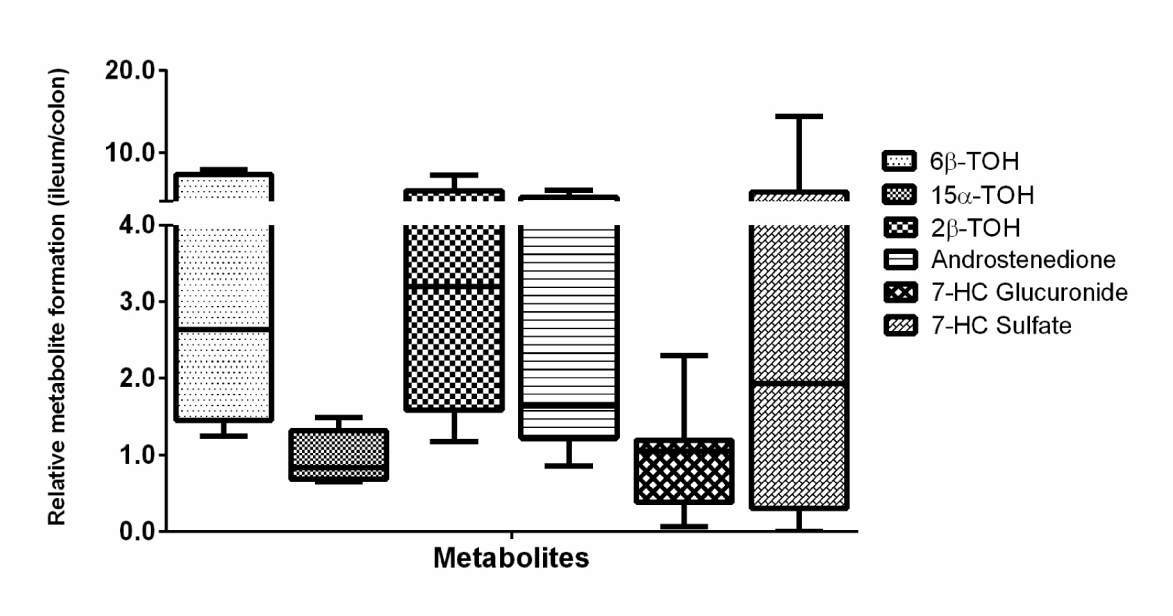

Figure 5. Testosterone and 7-Hydroxycoumarin metabolite formation in human ileum and colon slices (n=5-7). Data are expressed as mean +/- range.

Correlation between phase I and phase II metabolism

Finally, to understand better the relationship between phase I and phase II metabolism, we investigated the correlation between all the studied metabolites.

As demonstrated in **Figure 7**, we found a strong positive correlation in the ileum between 2β-TOH and 6β-TOH, 7-HC glucuronide and 6β-TOH, 7-HC sulfate and 6β-TOH, 7-HC glucuronide and 2β-TOH, as well as 7-HC sulfate and 2β-TOH (**Supplementary Table S2**). There was also a strong positive correlation between 7-HC glucuronide and 15α-TOH, as well as 7-HC sulfate and 7-HC glucuronide in the colon (**Supplementary Table S2**). However, we did not observe a correlation between the metabolic activity of phase I and phase II enzymes in matched slices (**Supplementary Figures S1 and S2**).

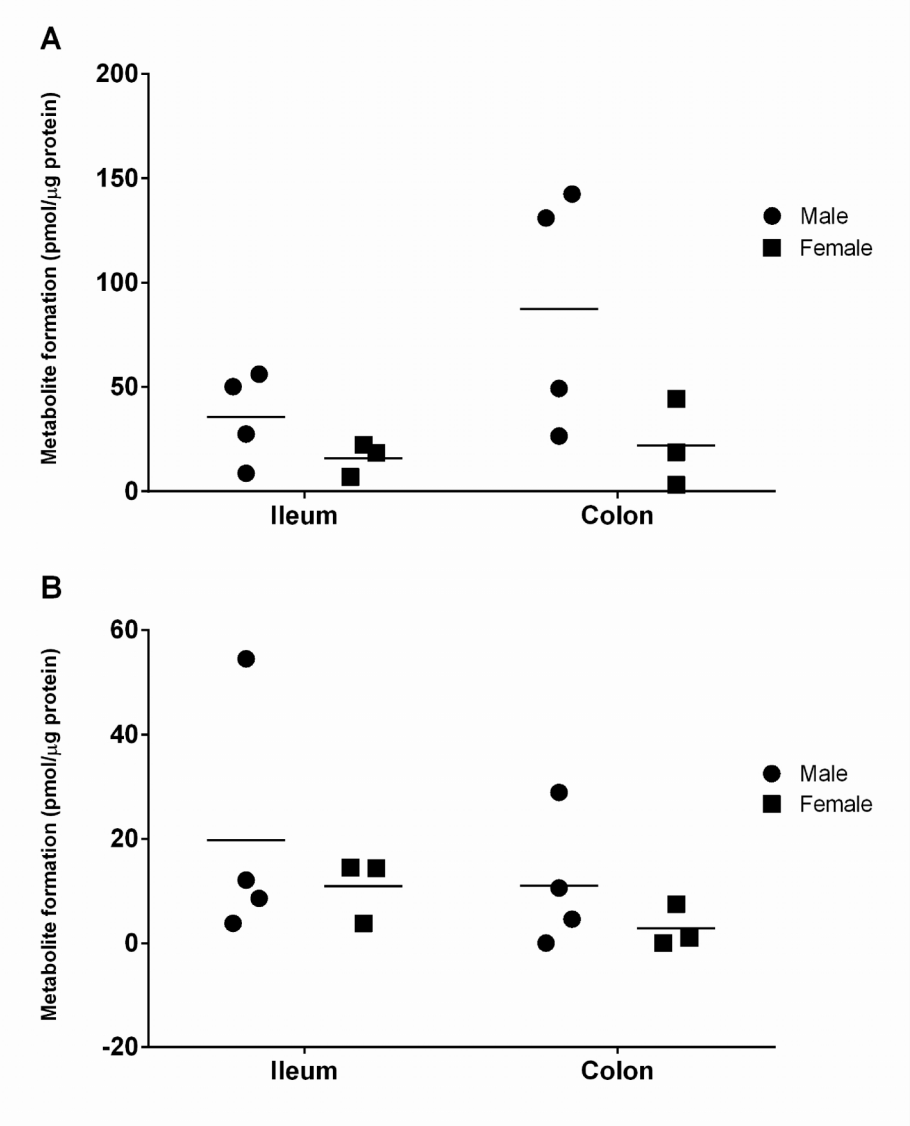

Figure 6. Sex differences on phase II metabolism of human ileum and colon slices: (A) 7-HC Glucuronide; (B) 7-HC Sulfate. Data are expressed as mean +/- range.

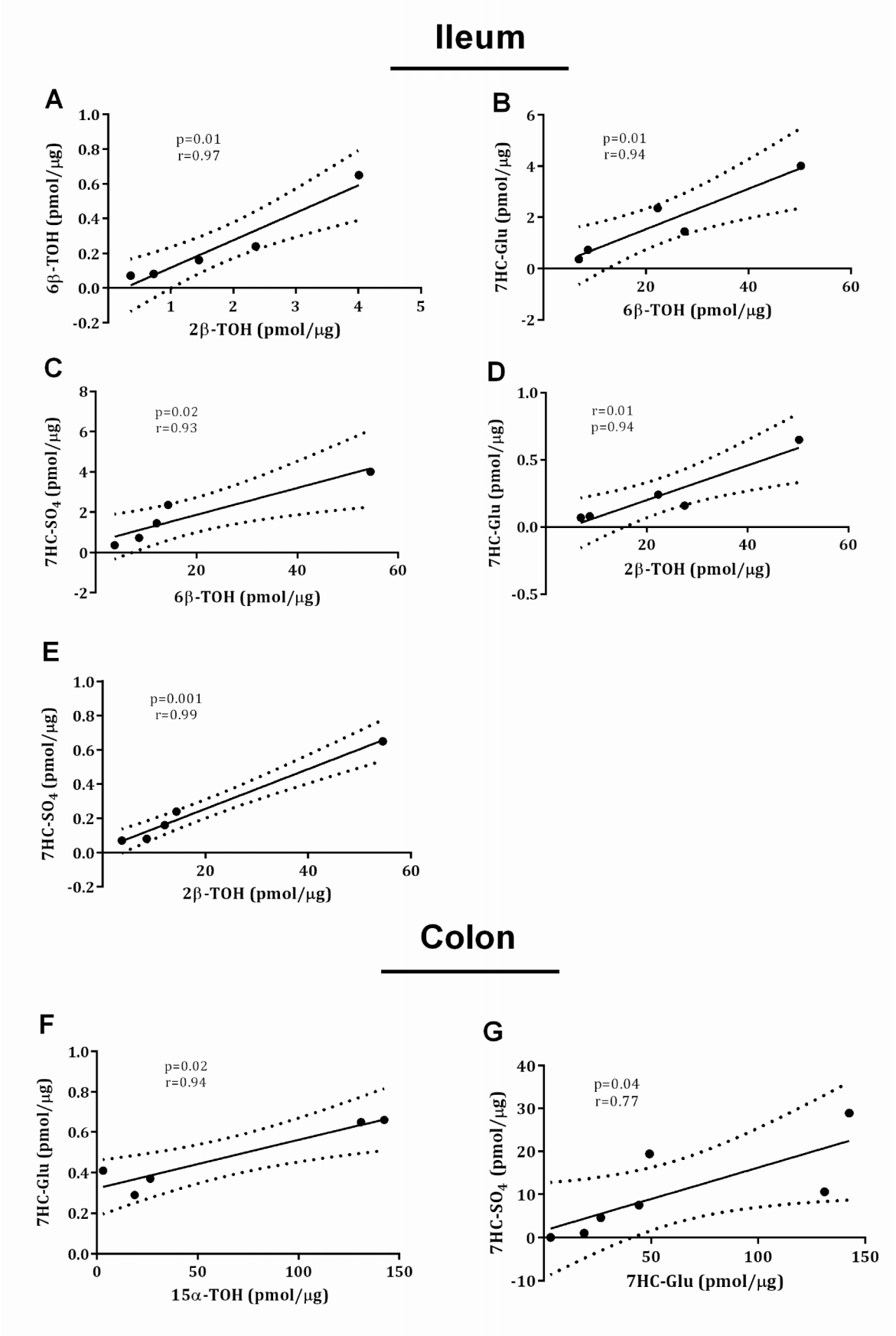

Figure 7. Correlations of phase I and phase II metabolite formation: in the ileum (A-E) and colon (F-G), n=5-7. $p<0.05$, $r>+/-0.5$.

DISCUSSION

The intestines fulfill an important role in drug metabolism, and it is known that the expression levels of the involved enzymes differ along the intestinal tract. However, studies in humans are lacking, especially with regards to intraindividual differences in regional metabolism. Here, we evaluated the differences in phase I and II metabolism using matched ileum and colon hPCIS.

Our results demonstrated that the ATP level, as a marker of viability, increased after 3 h of culture and remained elevated for 24 h in both human ileum and colon PCIS. A similar observation was previously made using rat PCIS.[15] In addition, we found no correlation between ATP levels and the formation of phase I and phase II metabolites, in both ileum and colon (**Supplementary Figures S3 and S4**). This result emphasizes that the viability of PCIS had no impact on the metabolic activity of phase I and phase II enzymes.

A unique aspect of this study was the fact that we were able to use matched ileum and colon hPCIS. This allowed us to show that there is no correlation between the metabolic rate of phase I and phase II enzymes in matched slices (**Supplementary Figures S1 and S2**). This strongly suggests that the regional drug metabolism capacity is differently regulated within one human being. Furthermore, pharmacokinetics and bioavailability of drugs are greatly affected in patients with a short small bowel following gastro-intestinal surgery, and the changes will vastly differ between patients.[29,30] Studies with matched hPCIS can aid in the identification of suitable therapies for this patient population.

We demonstrated that testosterone metabolism mostly occurred in the ileum. This result is in line with a previous study showing that protein levels of CYP3A, which exhibits testosterone 6β-hydroxylase activity,[31] is highest in the proximal region of the intestine and declines in the distal part.[21,32–34] In addition, other studies revealed that CYP3A and CYP2C are the major intestinal CYPs, accounting for approximately 80% and 18%, respectively, of all CYPs.[19,35] In line with our findings, Thelen and Dressman also showed that CYP3A content is generally higher in the proximal region of the intestine.[35]

Our results further demonstrated that metabolism of 7-HC mostly took place in the colon. These findings corroborate previous works showing that UGTs are primarily

found in the colon.[36,37] In contrast, Drozdzik *et al*. has reported that UGT1A1 was the most abundant UGT in jejunum, compared to other intestinal segments.[21] However, they only measured mRNA level and protein content, not enzyme activity.

Previously, the metabolic activity in intestinal slices and intestinal rat microsomes has been compared. The results showed that the metabolic rates in intestinal slices was significantly higher for all substrates studied (including TT, 3- to 29-fold) compared with the microsomes.[7] In addition, in agreement with our findings, Ho *et al.* described that CYP3A4 (TT as a substrate) was the most active enzymes in cryopreserved enterocytes from human small intestines followed by UGT and SULT (7-HC as a substrate), respectively.[38]

Furthermore, we also discovered a strong correlation between several products formed by CYP3A (e.g. 2β-TOH and 6β-TOH),[31,39] CYP2A (15α-TOH),[13] UGT1A (7-HC glucuronide)[16] and SULT1 (7-HC sulfate)[16] in both the ileum and colon. These results suggest that there might be a shared regulatory mechanism between phase I and phase II enzymes. Indeed, work by Xie and colleagues demonstrated that the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are involved in the regulation of CYP3A and CYP2B,[40] as well as specific UGT1A isoforms.[41] In addition, Maglich *et al*. has shown that CAR regulates CYP2A6 and SULT1.[42] Thus, it is clear that several signaling pathways can regulate both phase I and phase II enzymes.

We also observed that the rate of phase II metabolism was higher in male PCIS as compared to female PCIS. To the best of our knowledge, sex differences in human intestinal metabolism have not been reported before. However, using human liver microsomes, Court *et al.* previously established that acetaminophen metabolism, catalyzed by UGT1A1, UGT1A6, and UGT1A9, was higher in males as compared to females.[43] Men also show a higher rate of clearance of several benzodiazepines (*e.g.* diazepam, chlordiazepoxide, and olanzapine), which is catalyzed by CYP1A2. Furthermore, men more rapidly clear chloroxazone, a CYP2E1 substrate.[44] Also, it is clear that endogenous sex hormones can impact hepatic enzyme activity resulting in sex differences in pharmacokinetics.[44] However, Miyauchi *et al.* reported that there was no significant difference between men and women in the expression level of CYPs and UGTs in human jejunal tissues.[45] In addition, Her *et al.* described a large variation in the jejunal expression of SULT1E1 and SULT2A1 independent of gender or age.[46]

Thus, more research is needed to characterize fully sex-based differences in intestinal drug metabolism.

CONCLUSION

PCIS is a promising model to investigate intestinal drug metabolism. Based on this study, it can be concluded that there is a significant difference in the rate of phase I and II metabolism in the ileum and the colon. We demonstrated that phase I metabolism predominantly occurs in ileum PCIS, while phase II metabolism mostly takes place in colon PCIS. Moreover, our study revealed that PCIS could be used to investigate sex differences in drug metabolism.

ACKNOWLEDGMENTS

This work was supported by *De Nederlandse organisatie voor gezondheidsonderzoek en zorginnovatie* (ZonMw) – The Netherlands [Grant 114025003].

REFERENCES

1. Doherty, M. M. & Charman, W. N. The mucosa of the small intestine. *Clin. Pharmacokinet.* **41**, 235–253 (2002).
2. Ding, X. & Kaminsky, L. S. Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu. Rev. Pharmacol. Toxicol.* **43**, 149–173 (2003).
3. Wang, D. Q.-H., Tazuma, S., Cohen, D. E. & Carey, M. C. Feeding natural hydrophilic bile acids inhibits intestinal cholesterol absorption: studies in the gallstone-susceptible mouse. *Am. J. Physiol. Liver Physiol.* **285**, G494–G502 (2003).
4. Von Richter, O. *et al.* Determination of in vivo absorption, metabolism, and transport of drugs by the human intestinal wall and liver with a novel perfusion technique. *Clin. Pharmacol. Ther.* **70**, 217–227 (2001).
5. Glaeser, H. *et al.* Impact of concentration and rate of intraluminal drug delivery on absorption and gut wall metabolism of verapamil in humans. *Clin. Pharmacol. Ther.* **76**, 230–238 (2004).
6. Chhabra, R. S. Intestinal absorption and metabolism of xenobiotics. *Environ.*

Health Perspect. **33**, 61–69 (1979).

7. Martignoni, M., Groothuis, G. & De Kanter, R. Comparison of mouse and rat cytochrome P450-mediated metabolism in liver and intestine. *Drug Metab. Dispos.* **34**, 1047–1054 (2006).
8. de Waziers, I., Cugnenc, P. H., Yang, C. S., Leroux, J. P. & Beaune, P. H. Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J. Pharmacol. Exp. Ther.* **253**, 387–394 (1990).
9. Kim, S. *et al.* PubChem substance and compound databases. *Nucleic Acids Res.* **44**, D1202–D1213 (2016).
10. De Kanter, R. *et al.* Drug-metabolizing activity of human and rat liver, lung, kidney and intestine slices. *Xenobiotica* **32**, 349–362 (2002).
11. Man, H. C., Skipper, P. L., Wishnok, J. S. & Tannenbaum, S. R. Characterization of testosterone 11β-hydroxylation catalyzed by human liver microsomal cytochromes P450. *Drug Metab. Dispos.* **33**, 714–718 (2005).
12. Arlotto, M. P., Trant, J. M. & Estabrook, R. W. Measurement of steroid hydroxylation reactions by high-performance liquid chromatography as indicator of P450 identity and function. *Methods Enzymol.* **206**, 454–462 (1991).
13. Honkakoski, P. & Negishi, M. The structure, function, and regulation of cytochrome P450 2A enzymes. *Drug-Metab-Rev* **29**, 977–996 (1997).
14. Van De Kerkhof, E. G., De Graaf, I. A. M., De Jager, M. H., Meijer, D. K. F. & Groothuis, G. M. M. Characterization of rat small intestinal and colon precision-cut slices as an in vitro system for drug metabolism and induction studies. *Drug Metab. Dispos.* **33**, 1613–1620 (2005).
15. van de Kerkhof, E. G., de Graaf, I. A. M., de Jager, M. H. & Groothuis, G. M. M. Induction of phase I and II drug metabolism in rat small intestine and colon in vitro. *Drug Metab. Dispos.* **35**, 898–907 (2007).
16. Wang, Q. *et al.* Inter-species comparison of 7-hydroxycoumarin glucuronidation and sulfation in liver S9 fractions. *Vitr. Cell. Dev. Biol. - Anim.* **42**, 8–12 (2006).
17. Gröer, C. *et al.* Absolute protein quantification of clinically relevant cytochrome P450 enzymes and UDP-glucuronosyltransferases by mass spectrometry-based targeted proteomics. *J. Pharm. Biomed. Anal.* **100**, 393–401 (2014).

18. Busch, D., Fritz, A., Partecke, L. I., Heidecke, C. D. & Oswald, S. LC–MS/MS method for the simultaneous quantification of intestinal CYP and UGT activity. *J. Pharm. Biomed. Anal.* **155**, 194–201 (2018).
19. Nakamura, K. *et al.* Large-scale multiplex absolute protein quantification of drug-metabolizing enzymes and transporters in human intestine, liver, and kidney microsomes by SWATH-MS: Comparison with MRM/SRM and HR-MRM/PRM. *Proteomics* **16**, 2106–2117 (2016).
20. Emoto, C. *et al.* Characterization of cytochrome P450 enzymes involved in drug oxidations in mouse intestinal microsomes. *Xenobiotica* **30**, 943–953 (2000).
21. Drozdzik, M. *et al.* Protein Abundance of Clinically Relevant Drug-Metabolizing Enzymes in the Human Liver and Intestine: A Comparative Analysis in Paired Tissue Specimens. *Clin. Pharmacol. Ther.* **104**, 515–524 (2018).
22. Peters, S. A., Jones, C. R., Ungell, A. L. & Hatley, O. J. D. Predicting Drug Extraction in the Human Gut Wall: Assessing Contributions from Drug Metabolizing Enzymes and Transporter Proteins using Preclinical Models. *Clinical Pharmacokinetics* **55**, 673–696 (2016).
23. Van De Kerkhof, E. G., De Graaf, I. A. M., Ungell, A. L. B. & Groothuis, G. M. M. Induction of metabolism and transport in human intestine: Validation of precision-cut slices as a tool to study induction of drug metabolism in human intestine in vitro. *Drug Metab. Dispos.* **36**, 604–613 (2008).
24. Poosti, F. *et al.* Precision-cut kidney slices (PCKS) to study development of renal fibrosis and efficacy of drug targeting ex vivo. *Dis. Model. Mech.* **8**, 1227–1236 (2015).
25. Pham, B. T. *et al.* Precision-cut rat, mouse, and human intestinal slices as novel models for the early-onset of intestinal fibrosis. *Physiol. Rep.* **3**, (2015).
26. De Graaf, I. A. M. *et al.* Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. *Nature Protocols* **5**, 1540–1551 (2010).
27. Van De Kerkhof, E. G. *et al.* Innovative methods to study human intestinal drug metabolism in vitro: Precision-cut slices compared with Ussing chamber preparations. *Drug Metab. Dispos.* **34**, 1893–1902 (2006).
28. De Kanter, R. *et al.* A new technique for preparing precision-cut slices from small

intestine and colon for drug biotransformation studies. *J. Pharmacol. Toxicol. Methods* **51**, 65–72 (2005).

29. Titus, R., Kastenmeier, A. & Otterson, M. F. Consequences of gastrointestinal surgery on drug absorption. *Nutrition in Clinical Practice* **28**, 429–436 (2013).
30. Severijnen, R., Bayat, N., Bakker, H., Tolboom, J. & Bongaerts, G. Enteral drug absorption in patients with short small bowel: A review. *Clinical Pharmacokinetics* **43**, 951–962 (2004).
31. Emoto, C. *et al.* Use of everted sacs of mouse small intestine as enzyme sources for the study of drug oxidation activities in vitro. *Xenobiotica* **30**, 971–982 (2000).
32. Paine, M. F. *et al.* Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J. Pharmacol. Exp. Ther.* **283**, 1552–1562 (1997).
33. McKinnon, R. A. *et al.* Characterisation of CYP3A gene subfamily expression in human gastrointestinal tissues. *Gut* **36**, 259–67 (1995).
34. Xie, F., Ding, X. & Zhang, Q. Y. An update on the role of intestinal cytochrome P450 enzymes in drug disposition. *Acta Pharmaceutica Sinica B* **6**, 374–383 (2016).
35. Dressman, J. B. & Thelen, K. Cytochrome P450-mediated metabolism in the human gut wall. *J. Pharm. Pharmacol.* **61**, 541–558 (2009).
36. Pacifici, G. M. *et al.* Profile of drug-metabolizing enzymes in human ileum and colon. *Pharmacology* **38**, 137–145 (1989).
37. Zhang, H. *et al.* Quantitative characterization of UDP-glucuronosyltransferase 2B17 in human liver and intestine and its role in testosterone first-pass metabolism. *Biochem. Pharmacol.* **156**, 32–42 (2018).
38. Ho, M. C. D., Ring, N., Amaral, K., Doshi, U. & Li, A. P. Human enterocytes as an in vitro model for the evaluation of intestinal drug metabolism: Characterization of drug-metabolizing enzyme activities of cryopreserved human enterocytes from twenty-four donors. *Drug Metab. Dispos.* **45**, 686–691 (2017).
39. Vimercati, S., Büchi, M., Zielinski, J., Peduto, N. & Mevissen, M. Testosterone metabolism of equine single CYPs of the 3A subfamily compared to the human CYP3A4. *Toxicol. Vitr.* **41**, 83–91 (2017).
40. Xie, W. *et al.* Reciprocal activation of xenobiotic response genes by nuclear

receptors SXR/PXR and CAR. *Genes Dev.* **14**, 3014–3023 (2000).

41. Xie, W. *et al.* Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. *Proc. Natl. Acad. Sci.* **100**, 4150–4155 (2003).
42. Maglich, J. M. *et al.* Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes. *J. Biol. Chem.* **278**, 17277–17283 (2003).
43. Court, M. H. *et al.* Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. *J. Pharmacol. Exp. Ther.* **299**, 998–1006 (2001).
44. Waxman, D. J. & Holloway, M. G. Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes. *Mol. Pharmacol.* **76**, 215–228 (2009).
45. Miyauchi, E. *et al.* Quantitative Atlas of Cytochrome P450, UDP-Glucuronosyltransferase, and Transporter Proteins in Jejunum of Morbidly Obese Subjects. *Mol. Pharm.* **13**, 2631–2640 (2016).
46. Her, C., Szumlanski, C., Aksoy, I. A. & Weinshilboum, R. M. Human jejunal estrogen sulfotransferase and dehydroepiandrosterone sulfotransferase Immunochemical characterization of individual variation. *Drug Metab. Dispos.* **24**, 1328–1335 (1996).

SUPPLEMENTARY DATA

TABLE S1. Metabolic rates during 3 h of incubation in small intestinal (ileum) and colon slices (Data are expressed as mean +/- SEM, n=5-7).

Metabolite Formed	0-3 hours (pmol/µg protein)		Ratio (Ileum/Colon)
	Ileum	Colon	
Androstenedione	9.55 ± 2.27	4.34 ± 0.96	2.20
6β-TOH	1.78 ± 0.65	0.42 ± 0.05*	4.24
15α-TOH	0.33 ± 0.08	0.48 ± 0.08	0.69
2β-TOH	0.24 ± 0.11	0.06 ± 0.01	4.00
7-HC Glucuronide	27.16 ± 7.27	59.37 ± 20.85†	0.45
7-HC Sulfate	17.59 ± 6.34	10.29 ± 3.97	1.71

*Significantly different from ileum with $p < 0.05$
†Significantly different from 7-HC Sulfate colon with $p < 0.05$

TABLE S2. Correlations between phase I and phase II metabolite formation in the ileum (all data above the black box) and the colon (all data below the black box); n=5-7.

Correlation vs.	Andro	6β-TOH	15α-TOH	2β-TOH	7-HC Glucuronide	7-HC Sulfate
Andro		p=0.13 r=0.76	p=0.99 r=-0.01	p=0.27 r=0.62	p=0.34 r=0.54	p=0.32 r=0.56
6β-TOH	p=0.09 r=0.81		p=0.45 r=0.44	p=0.01 r=0.97	p=0.01 r=0.94	p=0.02 r=0.93
15α-TOH	p=0.74 r=0.21	p=0.54 r=0.37		p=0.50 r=0.40	p=0.26 r=0.63	p=0.39 r=0.50
2β-TOH	p=0.34 r=-0.55	p=0.97 r=0.63	p=0.93 r=0.06		p=0.01 r=0.94	p=0.001 r=0.99
7-HC Glucuronide	p=0.40 r=0.49	p=0.13 r=0.80	p=0.02 r=0.94	p=0.91 r=0.07		p=0.11 r=0.65
7-HC Sulfate	p=0.40 r=0.49	p=0.59 r=0.33	p=0.10 r=0.81	p=0.48 r=-0.42	p=0.04 r=0.77	

: Significantly different and strongly correlated (p<0.05, r>+/-0.5)

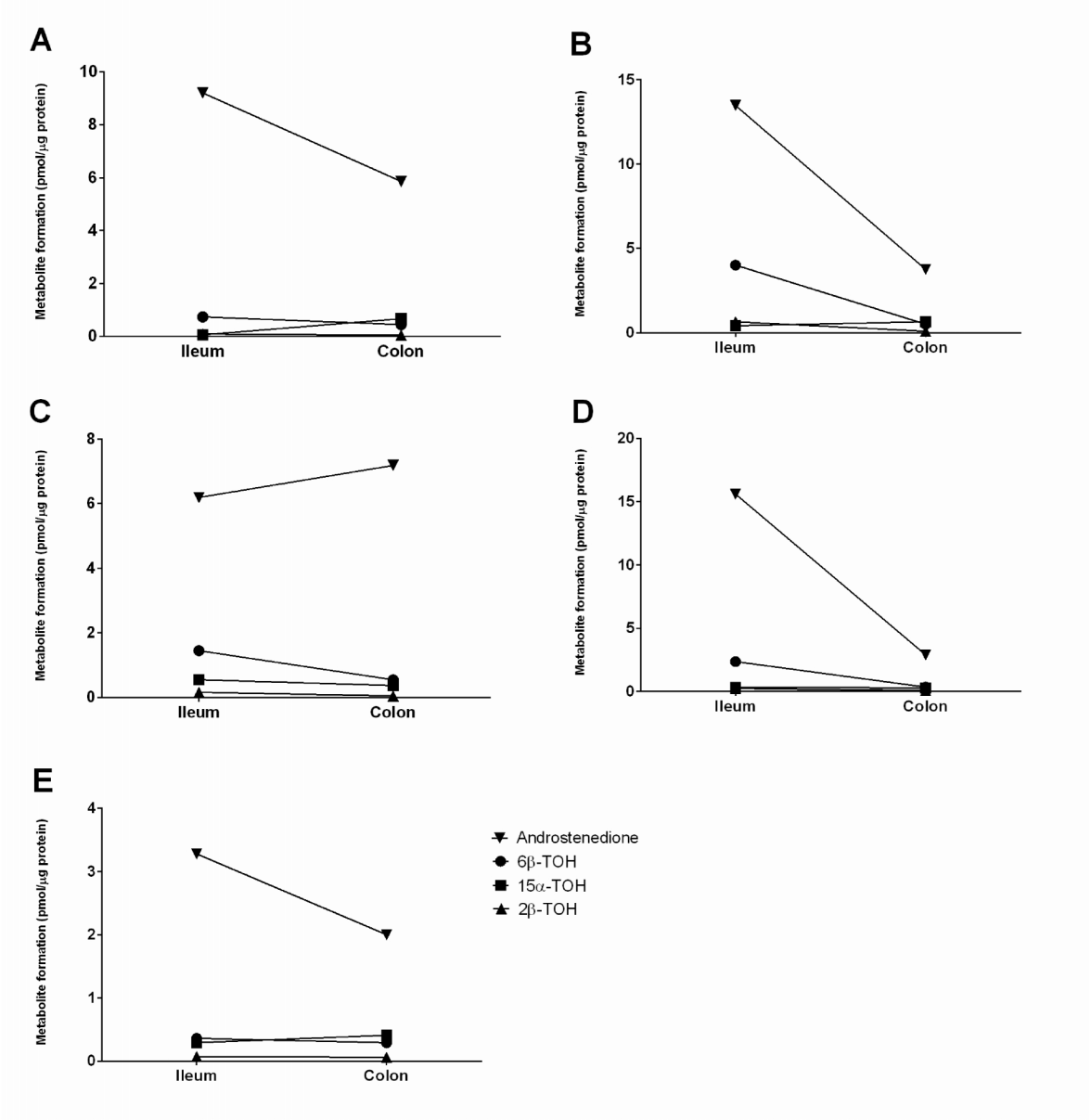

Figure S1. The metabolism rate of phase I in human ileum and colon PCIS: (A) Patient 1; (B) Patient 2; (C) Patient 3; (D) Patient 4; (E) Patient 5 (Each data was obtained triplicates; Zero metabolite formation due to the limit of detection of the HPLC).

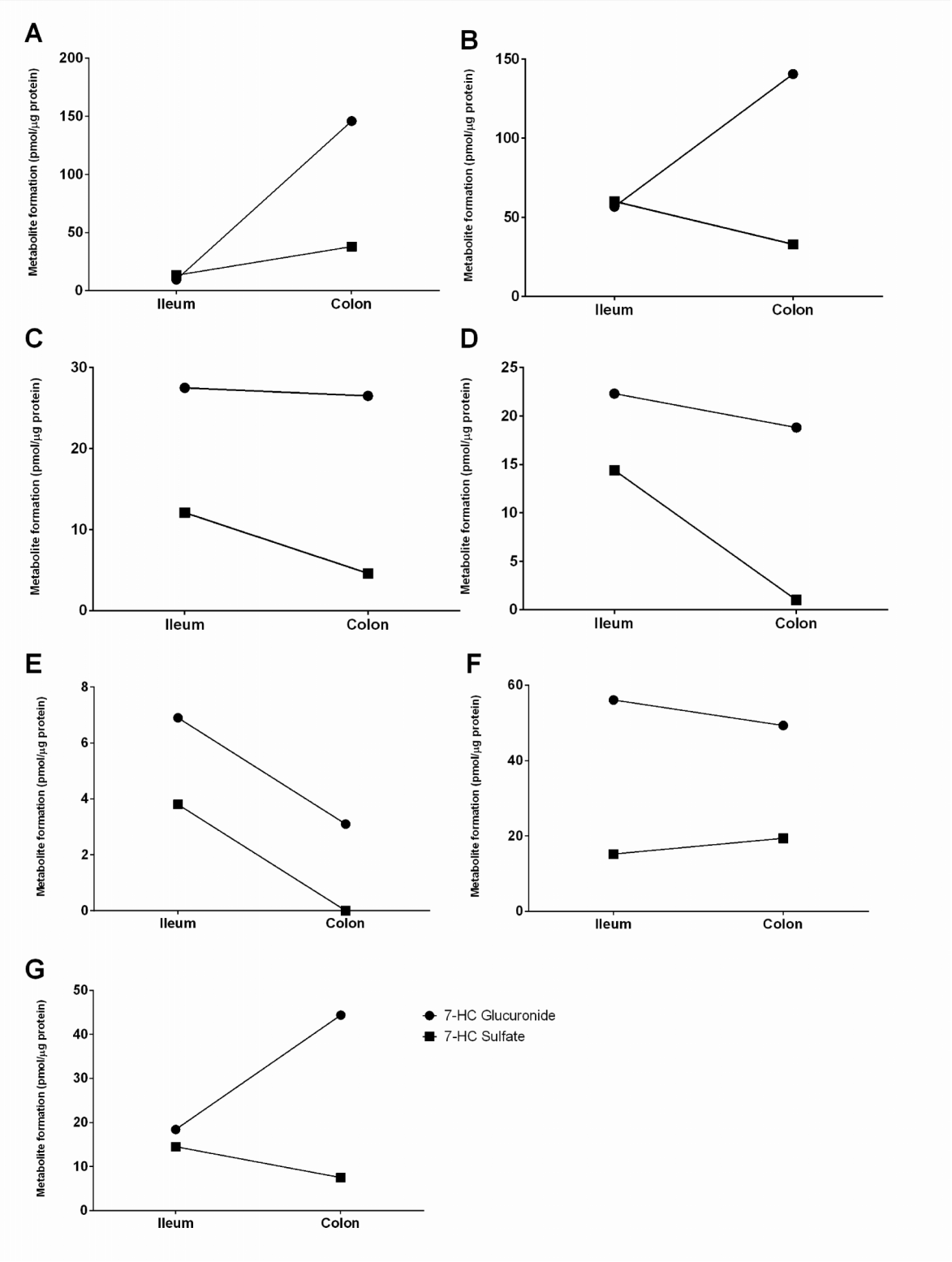

Figure S2. The metabolism rate of phase II in human ileum and colon PCIS: (A) Patient 1; (B) Patient 2; (C) Patient 3; (D) Patient 4; (E) Patient 5; (F) Patient 6; Patient 7 (Each data was obtained triplicates; Zero metabolite formation due to the limit of detection of the HPLC).

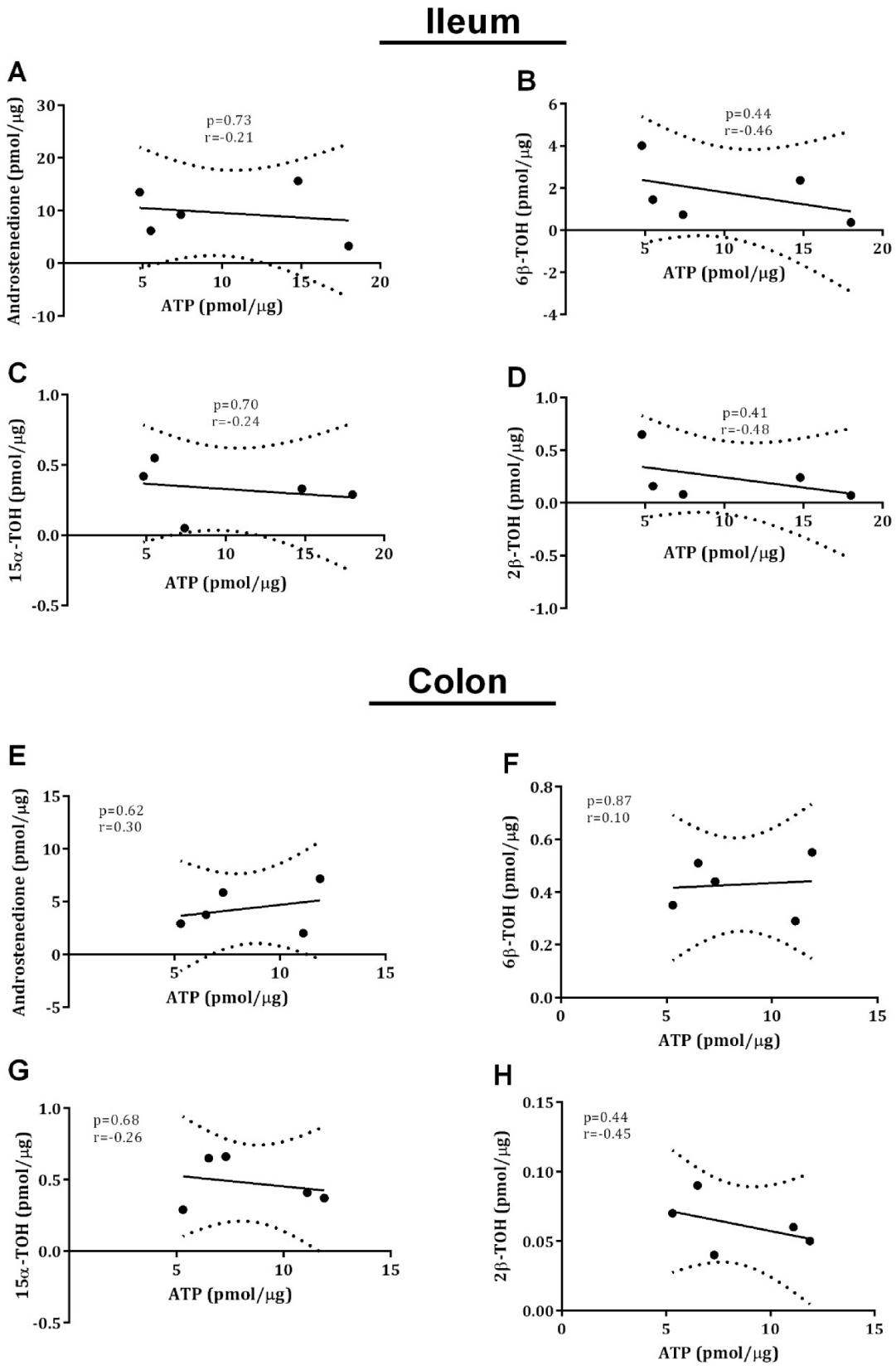

Figure S3. Correlations of ATP with the phase I metabolite formation: in the ileum (A-D) and colon (E-H), n=5.

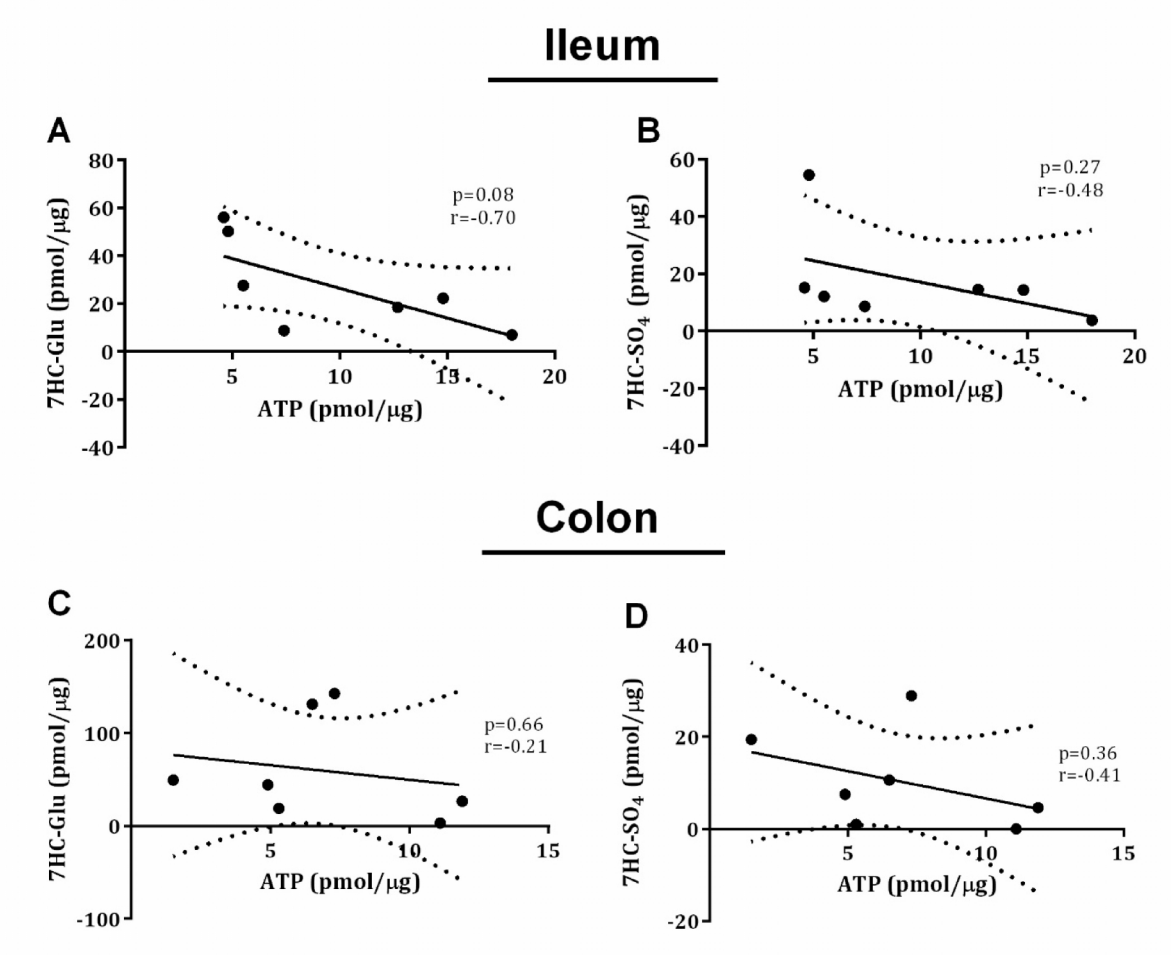

Figure S4. Correlations of ATP with the phase II metabolite formation: in the ileum (A and B) and colon (C and D), n=7.

Broken Chair, Geneva, Switzerland